(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 January 2002 (31.01.2002)

PCT

(10) International Publication Number
WO 02/08250 A2

(51) International Patent Classification[7]: C07K 7/00

(21) International Application Number: PCT/EP01/07929

(22) International Filing Date: 10 July 2001 (10.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/220,178 24 July 2000 (24.07.2000) US

(71) Applicant: **ZENTARIS AG** [DE/DE]; Weismüllerstrasse 45, 60314 Frankfurt (DE).

(72) Inventor: **DEGHENGHI, Romano**; Chesaux Dessus, CH-1264 St. Cergue (CH).

(74) Agent: **ZENTARIS AG**; Patent Department, Meissner Str. 35, 01445 Radebeul (DE).

(81) Designated States *(national)*: AU, BG, BR, BY, CA, CN, CO, CZ, EE, GE, HR, HU, ID, IL, IN, IS, JP, KG, KR, KZ, LT, LV, MK, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TR, UA, UZ, YU, ZA.

(84) Designated States *(regional)*: Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/08250 A2

(54) Title: GHRELIN ANTAGONISTS

(57) Abstract: Novel peptides are disclosed having antagonistic properties to the Growth Hormone releasing peptide known as Ghrelin. The new peptides are useful in decreasing the circulating levels of Growth Hormone in a mammal and have therapeutic value.

GHRELIN ANTAGONISTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of provisional application serial no. 60/220,178 filed July 13, 2000.

TECHNICAL FIELD

The invention relates to new growth hormone antagonists that can be administered to mammals to decrease the level of circulating growth hormone therein.

BACKGROUND

Ghrelin is a name for a family of related peptides of 27 or 28 amino acids which have been isolated in the stomach (M. Kojima et al., Nature, 402, 656-660, 1999; H. Hosoda et al., J. Biol. Chem., May 8, 2000) by a distinct cell type in rats and humans. It is further characterized by having an essential octanoyl ester attached to a serine residue. Ghrelins are known to be potent releasers of growth hormone (GH) in animals and man.

Synthetic variations of these peptides were investigated to determine whether improvements can be made on them, and the present invention results from that investigation.

SUMMARY OF THE INVENTION

It has surprisingly been found that novel peptides of the general formula:

Gly-Ser-Ser(Octanoyl)-Phe-A

where A is -OH, NH_2, Leu-Ser-Pro-Glu-X or -Ala-Lys-Leu-Gln-Pro-Arg-B

where B is -OH or NH_2 decrease, rather than increase the level of circulating

GH in mammals, presumably because these peptides antagonize the effect of the ghrelins. For this reason, these peptides are of value in normalizing or reducing elevated levels of growth hormone such as those found in acromegalic patients or in other tumor related overproduction GH.

DETAILED DESCRIPTION OF THE INVENTION

The instant peptides can be prepared by a number of synthetic methods such as exemplified in "Chemical Approaches to the Synthesis of Peptides and Proteins" by P. Lloyd-Williams et al., CRC Press, New York 1997, and similar works well known to peptide chemists.

These peptides are administered in aqueous solutions subcutaneously at doses of about 1 to 10mg/kg of body weight by bolus injection or by slow parenteral infusions. Also, these peptides may be administrated intranasally or intrapulmonary or via a sustained release formulation that includes a biodegradable polymer incorporating the peptide, or by other means well known to those of ordinary skill in the art, such as implantable osmotic pumps and the like.

EXAMPLES

The following examples illustrate the effectiveness of these novel peptides.

Example 1

By solid phase synthesis the following peptide was prepared:

Gly-Ser-Ser(Octanoyl)-Phe-Leu-Ser-Pro-Glu

Theoretical MW: 948.9 Found 948.9

Solubility in water: 0.7mg/ml

Purity by HPLC analysis: 97.8%

The peptide was injected subcutaneously in 10-day old rats at a dose of 300mg/kg along with a solvent control and Ghrelin, and the circulating GH determined at 15 minutes, as described in R. Deghenghi et al., Life Sciences 54, 1321-1328 (1994). The results were as follows:

Compounds	**GH ng/ml**
Solvent control	10.11 ± 1.6
Ghrelin (human)	139.80 ± 15.37
Peptide of Example 1	1.40 ± 0.32

This demonstrates that the present peptide antagonizes the effect of the ghrelins by reducing GH release to a level that is almost nil and much lower than the solvent control.

Example 2

By the same method of Example 1, the following tetradecapeptide was prepared:

Gly-Ser-Ser(Octanoyl)-Phe-Leu-Ser-Pro-Glu-Ala-Lys-Leu-Gln-Pro-Arg

Theoretical MW: 1642.7 Found: 1642.7

Solubility in water: 0.9 mg/ml

Purity by HPLC analysis: 95.0%

The peptide was administered to rats as described above in Example 1. The results were as follows:

Compound	GH ng/ml
Solvent control	10.11 ± 1.6
Ghrelin (human)	140 ± 15
Peptide of Example 2	7.00 ± 3.5

Again the inventive peptide is seen to antagonize the effect of the ghrelins by significantly reducing GH release to a level that is below that of the control.

Example 3

By the same method of Example 1, the following peptide was prepared:

Gly-Ser-Ser(Octanoyl)-Phe

Theoretical MW: 522.4 Found: 522.4

Solubility in water: insoluble

Purity by HPLC analysis: 95.6%

The peptide was administered to rats as described above in Example 1. The results were as follows:

Compound	GH ng/ml
Solvent control	10.1 ± 1.6

Ghrelin (human)	139.8 ± 15.4
Peptide of Example 3	7.7 ± 1.1

Yet again the inventive peptide antagonizes the effect of the ghrelins by significantly reducing GH release to a level that is below that of the control.

THE CLAIMS

What is claimed is:

1. A Ghrelin antagonist peptide of the formula:

Gly-Ser-Ser(Octanoyl)-Phe-A

where A is -OH, NH_2, Leu-Ser-Pro-Glu-B, or -Ala-Lys-Leu-Gln-Pro-Arg-B, where B is -OH or NH_2, wherein said peptide antagonizes the effect of ghrelins when administered to a mammal.

2. The peptide of claim 1 specifically as

Gly-Ser-Ser(Octanoyl)-Phe-Leu-Ser-Pro-Glu.

3. The peptide of claim 1 specifically as:

Gly-Ser-Ser(Octanoyl)-Phe-Leu-Ser-Pro-Glu-Ala-Lys-Leu-Gln-Pro-Arg.

4. The peptide of claim 1 specifically as:

Gly-Ser-Ser(Octanoyl)-Phe

5. A pharmaceutical composition comprising peptide of claim 1 in the form of a pharmaceutically acceptable salt.

6. The composition of claim 5 which further comprises a carrier.

7. The composition of claim 5 in the form of a sustained release formation or device for parenteral administration.

8. The peptide of claim 5 in the form of a pharmaceutically acceptable intranasal formulation.

9. The peptide of claim 5 in the form of a pharmaceutically acceptable inhalation formulation.

10. A method of normalizing elevated growth hormone levels in a mammal by administering to a mammal in need of such treatment an effective dose of at least one of the peptides of claim 1.

11. The method of claim 10 wherein the peptide is:

Gly-Ser-Ser(Octanoyl)-Phe-Leu-Ser-Pro-Glu.

12. The method of claim 11 wherein the peptide is:

Gly-Ser-Ser(Octanoyl)-Phe-Leu-Ser-Pro-Glu-Ala-Lys-Leu-Gln-Pro-Arg.

13. The method of claim 12 wherein the peptide is:

Gly-Ser-Ser(Octanoyl)-Phe.

14. The method of claim 10 wherein the peptide is administered as a sustained release formulation or through a device used for parenteral administration.

15. The method of claim 10 wherein the peptide is administered as a pharmaceutically acceptable intranasal formulation.

16. The method of claim 10 wherein the peptide is administered in a pharmaceutically acceptable inhalation formulation.

17. The method of claim 10 wherein the peptide is administered at a dosage of between about 1 and 10 mg/kg of body weight of the mammal.

5

18. The method of claim 10 wherein the peptide is administered to a mammal that is acromegalic.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 January 2002 (31.01.2002)

PCT

(10) International Publication Number
WO 02/008250 A3

(51) International Patent Classification[7]: C07K 14/60, 5/10, A61K 38/07, 38/25, A61P 5/04

(21) International Application Number: PCT/EP01/07929

(22) International Filing Date: 10 July 2001 (10.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/220,178 24 July 2000 (24.07.2000) US

(71) Applicant: **ZENTARIS AG** [DE/DE]; Weismüllerstrasse 45, 60314 Frankfurt (DE).

(72) Inventor: **DEGHENGHI, Romano**; Chesaux Dessus, CH-1264 St. Cergue (CH).

(74) Agent: **ZENTARIS AG**; Patent Department, Meissner Str. 35, 01445 Radebeul (DE).

(81) Designated States *(national)*: AU, BG, BR, BY, CA, CN, CO, CZ, EE, GE, HR, HU, ID, IL, IN, IS, JP, KG, KR, KZ, LT, LV, MK, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TR, UA, UZ, YU, ZA.

(84) Designated States *(regional)*: Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
22 August 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/008250 A3

(54) Title: GHRELIN ANTAGONISTS

(57) Abstract: Novel peptides are disclosed having antagonistic properties to the Growth Hormone releasing peptide known as Ghrelin. The new peptides are useful in decreasing the circulating levels of Growth Hormone in a mammal and have therapeutic value.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/07929

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/60 C07K5/10 A61K38/07 A61K38/25 A61P5/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE, CHEM ABS Data, SEQUENCE SEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KOJIMA MASAYASU ET AL: "Ghrelin is a growth-hormone-releasing acylated peptide from stomach" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 402, 9 December 1999 (1999-12-09), pages 656-660, XP002166936 ISSN: 0028-0836 cited in the application the whole document	
E	EP 1 197 496 A (KANGAWA KENJI) 17 April 2002 (2002-04-17) page 78, line 5	1,4-10, 13-18
E	WO 01 92292 A (BEDNAREK MARIA ;MERCK & CO INC (US)) 6 December 2001 (2001-12-06) claim 11	1,4-10, 13-18

[ ] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 June 2002	01/07/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Niemann, F

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 01/07929

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 10-18 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int onal Application No
PCT/EP 01/07929

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1197496 A	17-04-2002	AU 6023100 A	13-02-2001
		BR 0012688 A	16-04-2002
		EP 1197496 A1	17-04-2002
		WO 0107475 A1	01-02-2001
WO 0192292 A	06-12-2001	WO 0192292 A2	06-12-2001